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Purification and Identification of a Plasma Membrane Associated Electron Transport Protein from Maize (*Zea mays* L.) Roots

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ABSTRACT

Plasma membranes isolated from three-day-old maize (*Zea mays* L.) roots by aqueous two-phase partitioning were used as starting material for the purification of a novel electron transport enzyme. The detergent-solubilized enzyme was purified by dye-ligand affinity chromatography on Cibacron blue 3G-A-agarose. Elution was achieved with a gradient of 0 to 30 micromolar NADH. The purified protein fraction exhibited a single 27 kilodalton silver nitrate-stained band on sodium dodecyl sulfate polyacrylamide gel electrophoretograms. Staining intensity correlated with the enzyme activity profile when analyzed in affinity chromatography column fractions. The enzyme was capable of accepting electrons from NADPH or NADH to reduce either ferricyanide, juglone, duroquinone, or cytochrome c, but did not transfer electrons to ascorbate free-radical or nitrate. The high degree of purity of plasma membranes used as starting material as well as the demonstrated insensitivity to mitochondrial electron transport inhibitors confirmed the plasma membrane origin of this enzyme. The purified reductase was stimulated upon prolonged incubation with flavin mononucleotide suggesting that the enzyme may be a flavoprotein. Established effectors of plasma membrane electron transport systems had little effect on the purified enzyme, with the exception of the sulfhydryl inhibitor *p*-chloromercuriphenylsulfonate, which was a strong inhibitor of ferricyanide reducing activity.

It is now firmly established that eukaryotic cells contain endogenous electron transport enzymes integral to the PM.[2] While a role for these activities has not been established for many cell types, the localization of electron transport activities on the PM argues persuasively for their participation in transmembrane events at the cell surface. In root cells of nongraminaceous, monocotyledenous plants as well as dicotyledenous plants, the PM electron transport function is manifested as a transition metal-chelate reductase, responsible for the obligatory reduction of ferric chelates prior to uptake, and is induced by iron stress (4). Similarly, mammalian liver cells contain a PM differic transferrin reductase, also putatively linked to an iron uptake mechanism *via* transferrin receptors (24).

In the maize (*Zea mays* L.) root PM, multiple electron transport activities appear to comprise a complex electron transport system of components unique to the PM (6, 13). At least two separate enzymes, capable of transfer of electrons from pyridine nucleotides to ferricyanide and/or duroquinone, are measurable in PM fractions purified from maize roots. An ascorbate free-radical reductase also appears to be a separate component associated with the maize root PM (13). While recent progress has been made in purification and identification of plasma membrane electron transport enzymes (3, 11, 13), definitive proof of identity and localization of PM redox components is lacking in both plant and animal cells. We report here the isolation, characterization and molecular identification of a 27 kD electron transport protein of the maize root PM.

MATERIALS AND METHODS

Plant Material

Maize (*Zea mays* L.) seedlings were dark grown for 3 d and roots harvested as described (5).

Isolation of PM

Microsomal membranes were prepared from maize roots as described (5) with modifications (13). A 6.2% (w/w) aqueous polymer two-phase partitioning system was employed as described (5) for isolation of PM from maize roots. To increase yields of PM, 36 g phase systems were loaded with approximately 5 to 10 g of microsomal protein isolated from 300 to 500 g fresh weight of maize roots. Three separate phases were employed as described (5). Upper phase PM fractions were diluted, pelleted, resuspended and stored at −80°C as described (13). Stored PM preparations were diluted in and washed with 10 volumes of 0.5 M KCl in 15 mM Tris-Mes (pH 7.5), 10% (w/v) glycerol using 10 strokes of a Teflon[3]

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[2] Abbreviations: PM, plasma membrane(s); FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; FCR, ferricyanide reductase; DQR, duroquinone reductase; AFR, ascorbate free-radical reductase; AcPyAdP, 3-acetylpyridine adenine dinucleotide phosphate; PCMPS, *p*-chloromercuriphenylsulfonate; %T, percent total monomers (acrylamide + *N,N'*-methylene-bis-acrylamide).

[3] The mention of vendor or product does not imply that they are endorsed or recommended by U.S. Department of Agriculture over vendors of similar products not mentioned.

pestle homogenizer and repelleted at 113,000g_{max} for 45 min before solubilization.

Detergent Solubilization of PM

Stored PM fractions were pooled and solubilized with Triton X-100 at a detergent to protein ratio of 1.0 (w/w) as described (13), substituting 10% (w/v) glycerol for sucrose in the resuspension buffer. Protein inhibitors (10) were included as described (13). Purified Triton X-100 (Pierce Chemical Co., Rockford, IL), which had been purged of oxygen and stored under N_2, was used in the solubilization and purification procedures.

Dye-Ligand Affinity Chromatography

PM redox activities that had been solubilized with Triton X-100 at a detergent to protein ratio of 1.0 (w/w) were diluted with 4 volumes of 15 mM Tris-Mes (pH 7.0), 10% (w/v) glycerol, 100 μM DTT, 1 μM FAD, 1 μM FMN, and 0.10% (w/v) Triton X-100 (column buffer) and applied at a flow rate of 0.25 ml·min^{-1} to a 1.5 × 25 cm column of Cibacron blue 3G-A-agarose (Affi-Gel blue, 50-100 mesh, Bio-Rad, Rockville Centre, NY) which had been previously equilibrated with 10 bed volumes of column buffer. The column was subsequently washed with 5 bed volumes of column buffer at a flow rate of 0.5 ml·min^{-1} to remove unbound protein. Reductase activities were eluted with a 60 mL linear gradient of 0 to 30 μM NADH (measured by absorbance at 340 nm) in column buffer at a flow rate of 0.25 mL·min^{-1}. All procedures were conducted at 5°C in a cold box. Protein was measured in column fractions by the method of Markwell *et al.* (16) following precipitation with 6% (w/v) TCA plus 200 μg·ml^{-1} deoxycholate (1).

Enzyme Assays

NAD(P)H:FCR, NAD(P)H:DQR, and NAD(P)H:AFR were assayed as described (13), substituting 10% glycerol (w/v) for sucrose in the assay buffer and including 0.01% (w/v) Triton X-100. NAD(P)H:juglone (5-hydroxy-1,4-napthoquinone) reductase was measured as for NAD(P)H:DQR, substituting 100 μM juglone (prepared as 10 mM stock solution in ethanol) for duroquinone. NAD(P)H:Cyt *c* reductase was assayed in 15 mM Tris-Mes (pH 7.5), 10% (w/v) glycerol, 0.01% (w/v) Triton X-100 (assay buffer), using 160 μM NAD(P)H and 40 μM Cyt *c*. The absorbance of Cyt *c* was measured at 550 nm, and an extinction coefficient of 21.1 mM^{-1} cm^{-1} was used in calculations of NAD(P)H: Cyt *c* activity. NAD(P)H:nitrate reductase and NAD(P)H: thioredoxin reductase were measured at 340 nm, using 0.2 mM NAD(P)H and 2.0 mM KNO_3 or 100 μM (oxidized) thioredoxin in assay buffer, using an extinction coefficient for NAD(P)H-dependent assays of 6.22 mM^{-1} cm^{-1} in calculation of activities. Transhydrogenase activity was measured in assay buffer as the rate of reduction of the NADP analog AcPyAdP at 375 nm by NAD(P)H, using an extinction coefficient of 5.1 mM^{-1}·cm^{-1}. All assays were conducted at 30°C. Protein in PM fractions was measured using the method of Bradford (2). Adriamycin (doxorubicin), antimycin A, Cyt *c*, 2,4-D, duroquinone (tetramethylquinone), FAD, FMN, juglone (5-hydroxy 1,4 napthoquinone), NADH, NADPH, PCMPS and quinacrine were obtained from Sigma (Sigma Chemical Co., St. Louis, MO). Recombinant *Escherichia coli* thioredoxin was from Calbiochem (Calbiochem, Behring Diagnostics, La Jolla, CA).

SDS-PAGE

NADH-eluted fractions from Cibacron blue 3G-A-agarose chromatography were precipitated with TCA as described above for protein assay. TCA precipitates were washed in ice-cold 100% ethanol and reprecipitated. The pellets resulting from TCA and ethanol precipitation were resuspended in 3% w/v SDS, 25 mM DTT Laemmli sample buffer (12), and heated at 100°C for 5 min. Samples were applied to a 1.5 mm thick vertical polyacrylamide slab composed of a 7 to 15% T (w/v) linear gradient with a 4% T (w/v) stacking gel and electrophoresed at 25 mamp constant current. Silver nitrate staining was performed according to Merril *et al.* (17) or by manufacturer's instructions (Gelcode, Pierce Chemical Corp., Rockford, IL). Standard proteins of known mol wt (Bio-Rad) were included in separate lanes for mol wt estimations.

RESULTS

Purification and Identification of NAD(P)H:FCR/DQR

As described previously (13), dye-ligand affinity chromatography of a Triton X-100-solubilized maize root PM fraction on Cibacron blue 3G-A-agarose, exploiting biospecific elution with 100 μM NADH, produced an enzyme preparation capable of DQR and FCR activity, which was of a high degree of purity and which contained at least three silver nitrate-stained polypeptides following separation by SDS-PAGE. Subsequently, elution with a linear 0 to 30 μM NADH concentration gradient was employed to further resolve polypeptides responsible for electron transport catalysis. A well resolved peak of NADH-dependent reductase activity was eluted from Cibacron blue 3-GA-agarose at between approximately 5 and 10 μM NADH (Fig. 1A), as calculated by extrapolation between fractions at the initiation of the gradient and at 20 μM NADH. The elution profiles for the independently measured FCR and DQR activities were precisely coincidental.

SDS-PAGE analysis of the resultant affinity chromatography fractions demonstrated a single 27 kD polypeptide that was present in peak activity fractions in amounts which correlated in silver nitrate staining intensity with reductase activities (Fig. 1B). SDS-PAGE analyses of Coomassie plus silver nitrate- or enhanced silver nitrate-stained (Gelcode) polypeptides from TCA-precipitated peak enzyme column fractions obtained from repeated, identical experiments indicated the presence of minor contaminants of 56, 51, 17, and 16 kD which did not correlate in staining intensity with enzyme activity (data not shown).

The degree of purification of the resulting enzyme preparation (Table I) was roughly similar to that reported previously in an optimal experiment, which had employed elution with a single step concentration of 100 μM NADH from Cibacron

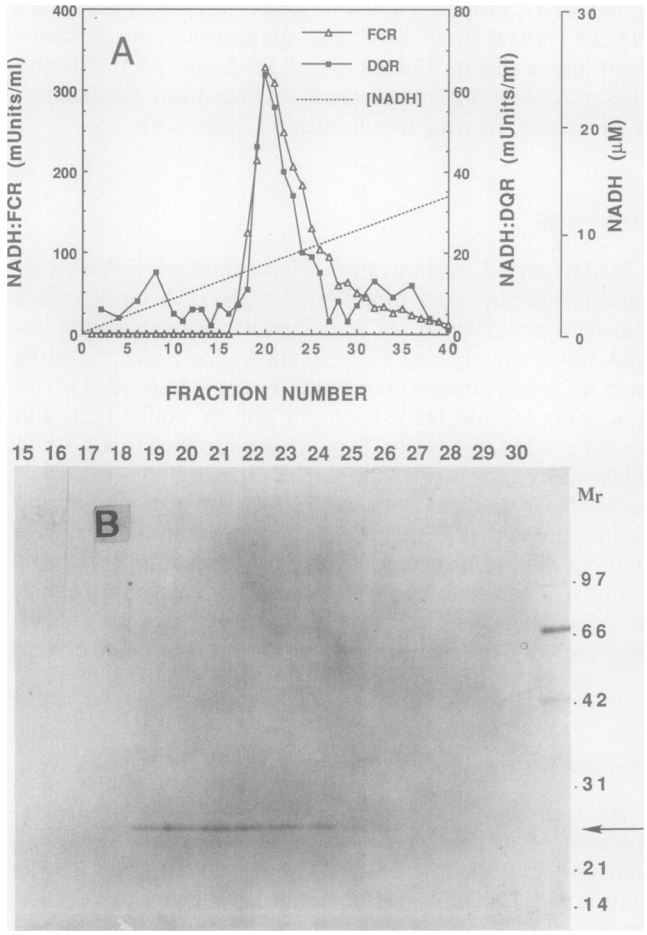

Figure 1. Purification of DQR and FCR by dye-ligand affinity chromatography. A, Elution of NADH:FCR and NADH:DQR from Cibacron blue 3G-A using a 60 mL, 0 to 30 μM NADH linear gradient, collecting 1 mL fractions. One milliunit of enzyme activity catalyzes 1 nmol·min^{-1} of substrate to product. B, SDS-PAGE analysis of peak NADH:FCR/DQR fractions eluted by NADH. Only that portion of the 0 to 30 μM NADH gradient (dashed line) which eluted enzyme activity (fractions 15–30) is shown. Mol wt markers in kD are indicated to the right of the figure. The arrow indicates the 27 kD protein eluted by NADH. See "Materials and Methods" for experimental details.

blue 3-GA-agarose loaded with Triton-solubilized maize root PM fraction (13). However, yields of DQR activity were improved under the refined gradient elution protocol. Recovery of total activities from the chromatography step were consistently between 90 and 100% for DQR and less than 30% for FCR in two separate experiments identical to that represented in Table I (data not shown). In addition, the ratio of FCR to DQR total activity was decreased approximately threefold from 15 in whole PM fractions to 5 in purified enzyme fractions. Thus, considerable FCR activity was likely resolved from the DQR during purification by the gradient elution scheme. The improvement of DQR yields over previous experiments (13) can be attributed to the inclusion of DTT in column buffers, as well as shortened chromatography protocols due to faster flow rates resulting from the use of a larger mesh size (50–100 mesh) of the stationary phase Cibacron blue 3-GA-agarose. Previous protocols utilized 100 to 200 mesh size agarose.

Characteristics of the Purified Enzyme

The purified reductase catalyzed the electron transfer from NADH or NADPH to either ferricyanide or duroquinone (Table II). The enzyme did not demonstrate a distinct preference for either of the reduced pyridine nucleotide electron donors using ferricyanide or duroquinone as the electron acceptor. This was in sharp contrast to ratios of duroquinone and ferricyanide reductase rates obtained using NADPH *versus* NADH in PM vesicles (13), where NADH was the preferred electron donor. This shift in electron donor specificity indicated the existence of several reductase species in the PM. Enzymatic rates measured in PM vesicles with NADH and NAD(P)H thus may represent the sum of activities from more than one species of DQR and FCR. In sharp contrast to the activities measured with duroquinone or ferricyanide, the activity of the purified enzyme measured with juglone as the electron acceptor demonstrated a distinct preference for NADPH as the electron donor.

The purified reductase catalyzed the NAD(P)H-dependent reduction of Cyt *c* at approximately 10% of the rate of duroquinone reduction, while ascorbate free-radical was not an acceptor substrate. The absence of ascorbate free-radical reductase activity in preparations of purified DQR confirmed our earlier observations (13) that the AFR was resolved from the DQR by affinity chromatography on Cibacron blue 3G-A-agarose.

We investigated the possibility that the purified enzyme could be capable of pyridine nucleotide-dependent nitrate reductase activity, because nitrate reductase from corn roots is a flavoprotein (7) which has been purified by dye-ligand affinity chromatography using NADH elution with Cibacron blue 3G-A-agarose (21) and is capable of NADH-dependent ferric chelate reduction (8). Nitrate reductase activity could not be detected in freshly isolated maize root PM fractions (DG Luster, TJ Buckhout, unpublished data). We further found that nitrate reductase activity was not detected in purified enzyme preparations (Table II). Similarly, thioredoxin reductase activity was investigated, as sulfhydryl reductases have been suggested as a potential mechanism for control of specific membrane transport and bioenergetic functions. Under the conditions tested, neither the purified enzyme (Table II) nor Triton-solubilized PM fractions (data not shown) contained measurable NAD(P)H:thioredoxin reductase activity. Transhydrogenase activity, measured as the rate of reduction of the $NADP^+$ analog $AcPyAdP^+$ by NAD(P)H, was also not detectable in Triton-solubilized PM (data not shown) or purified enzyme preparations (Table II).

Information on the nature of the reductase-catalyzed NADH:ferricyanide reaction was obtained using some common electron transport enzyme inhibitors and cofactors. FCR activity measured at 420 nm was compared, as many of the substances tested were found to interfere with measurements of absorbance of NADH oxidation at 340 nm using a split beam spectrophotometer. Neither antimycin A nor KCN, established inhibitors of mitochondrial electron transport, had a significant effect on NADH:ferricyanide reductase activity

Table I. *Purification of NADH-Dependent Maize Root PM Electron Transport Activities by Dye-Ligand Affinity Chromatography on Cibacron Blue 3-GA-Agarose*

The Triton supernatant remaining from centrifugation of Triton X-100 solubilized PM proteins and defined the starting point for affinity chromatography. Yield is defined as the percent of purified enzyme eluted from the affinity column with NADH, while recoveries include the amount of enzyme activity which did not bind to the affinity matrix, each based upon the total activity (Triton supernatant) applied to the column. Results are from a single, typical experiment ($n = 3$).

Fraction	Total Activity[a]	Total Protein	Specific Activity[b]	Purification	Yield	Recovery
		mg		*-fold*	%	
A. NADH: FCR						
Triton supernatant	38	40	0.95		100	100
NADH eluate	4.3	0.02	203	214	11.3	27.7
B. NADH: DQR						
Triton supernatant	1.2	40	0.03		100	100
NADH eluate	0.9	0.02	43	1433	75	108

[a] μmol·min^{-1}. [b] μmol (min·mg protein)$^{-1}$.

Table II. *Donor and Acceptor Substrate Specificity of the Purified PM Redox Protein*

Assays were performed in assay buffer containing 0.01% (w/v) Triton X-100, at 30°C. Pyridine nucleotides were used at 160 μM, Cyt *c* at 0.5 mg ml^{-1}, thioredoxin (oxidized form) at 100 μM and nitrate at 10 mM. Values represent means from two replications made on at least two independent enzyme preparations.

Acceptor	Donor		NADPH/NADH
	NADH	NADPH	
	μmol·min^{-1}·mg^{-1}		ratio
Ferricyanide	120	147	1.23
Juglone	86	142	1.65
Duroquinone	36	28	0.78
Cyt *c*	2.8	3.1	1.11
Ascorbate free-radical	ND[a]	ND	
Nitrate	ND	ND	
Thioredoxin	NT[b]	ND	
AcPyAdP	ND	ND	

[a] Activity not detected using *circa* 10-fold the amount of enzyme needed to detect FCR in the spectrophotometric assay. [b] Not tested.

Table III. *Effectors of NADH:FCR Activity of the Purified PM Redox Protein*

The compounds listed were incubated at the indicated concentration for 3 min in the 1 mL assay volume with purified enzyme at 25°C (PCMPS only) or 0°C and immediately assayed for NADH:FCR activity, compared to controls incubated with buffer or ethanol. Control rates ranged from 80–120 μmol ferricyanide reduced·min^{-1}·mg protein^{-1}.

Effector	Concentration	Enzyme Activity
	μM	% *control rate*
Antimycin A	20	96
KCN	500	92
PCMPS	1	93
	10	46
	100	35
Quinacrine	50	130
Actinomycin D	50	111
Adriamycin	10	83
	50	76
2,4-D	10	96
FAD[a]	1	116
FMN[a]	1	320

[a] A preparation of enzyme prepared without inclusion of flavins in the affinity chromatography column buffer was incubated with the indicated flavin for 60 min on ice and subsequently assayed for NADH:FCR activity. Control rates averaged 40 μmol ferricyanide reduced·min^{-1}·mg protein^{-1} in enzyme preparations isolated without flavins in column buffers.

of the purified reductase (Table III). Insensitivity to KCN, which is a metal ion chelator, further suggested that the enzyme was neither a heme-bearing peroxidase nor a metalloenzyme. The flavin biosynthesis antagonist quinacrine (atebrin), was slightly stimulatory at 50 μM, as previously observed for a NADH:ferricyanide reductase in purified castor bean glyoxysomal membranes (14). *Para*-nitrophenyl acetate, an inhibitor of FCR activity in soybean hypocotyl PM vesicles (18, 22), did not inhibit FCR activity in freshly isolated PM vesicles (DG Luster, CJ Robinson, TJ Buckhout, unpublished data) and was therefore not tested on purified enzyme preparations. Neither actinomycin D, reported to inhibit soybean hypocotyl PM FCR activity (18), nor the auxin analog 2,4-D, reported to stimulate an NADH oxidase partially purified from soybean hypocotyl PM (3, 18), had an effect on FCR activity of the purified maize root PM enzyme (Table III). Of the compounds tested, only the sulfhydryl antagonist PCMPS was a potent inhibitor of FCR activity with a half-maximal inhibition at 10 μM (Table III). The inhibition of PCMPS suggested the presence of sulfhydryl groups on at least one active site of the enzyme. PCMPS also inhibited DQR activity in detergent-solubilized PM vesicles (data not shown).

Adriamycin (doxorubicin), reported as an inhibitor of electron transport in plasma membranes isolated from mouse liver (23) and soybean hypocotyls (18), was without consistent effect on the FCR activity of the purified enzyme (Table III). Adriamycin was found to affect FCR activity only in prepa-

rations of the purified reductase which contained surplus free FAD and FMN (1 μM each) from column buffers. This suggested to us that a reduction of adriamycin with concomitant shift in adriamycin absorbance between 410 and 450 nm had occurred, leading to interference with the measurement of reduction of ferricyanide (data not shown). Adriamycin has been reported to undergo one electron redox shifts involving paramagnetic species *in vivo*, associated with anticancer activity in mammalian systems (9). The purified reductase may have reduced adriamycin directly, as erythrocyte membranes were reported to reduce a related anthracycline antibiotic by a pyridine nucleotide-dependent mechanism (20).

Incubation of the purified reductase with flavins was used to determine the specificity, if any, of the purified reductase for a potential flavin cofactor. FCR was stimulated approximately threefold when purified enzyme, which had been isolated without inclusion of flavins in the column buffer, was incubated for 60 min at 0°C with 1 μM FMN (Table III). No such stimulation was observed with FAD, and shorter incubation times with FMN were ineffective in stimulation of NADH:FCR activity.

DISCUSSION

Electrophoretic analysis of peak enzyme fractions from dye-ligand affinity chromatography of Triton-solubilized maize root PM proteins has consistently identified a 27 kD protein which was capable of PM-associated pyridine nucleotide-dependent electron transport enzyme activity. Critical to the purification procedure was an extensive washing of the dye-bound reductase with a minimum of five column volumes of column run buffer, prior to the biospecific elution step. This washing step presumably removed nonspecifically bound proteins as well as proteins sharing Triton X-100 micelles with the specifically bound reductase. The shared-micelle proteins were thus exchanged into micelles in the mobile phase and washed free of the column. Columns loaded with solubilized PM and washed with less than five column bed volumes of column buffer exhibited a 40 kD polypeptide upon elution with NADH and subsequent SDS-PAGE in addition to the 27 kD polypeptide associated with reductase activity (data not shown). The 40 kD protein was previously suspected as a candidate for the reductase (13). It is unclear whether the 40 kD protein was responsible for a portion of the FCR activity resolved from the purified preparation by the refined gradient elution scheme and/or increased column washing, as extreme dilution of the 40 kD protein during the column washing and elution protocols precluded its recovery and analysis for redox activity.

The altered properties of the purified reductase as compared to those observed in whole PM vesicles are apparently the result of resolution of the enzyme from multiple activities present in whole PM. Evidence from previous chromatographic resolution of the various PM FCR activities (13) indicates that the shift in pyridine nucleotide specificity observed for FCR activity between whole PM (NADPH:NADH activity ratio = 0.47) (13) and purified (NADPH:NADH activity ratio = 1.23) (Table II) was due to the existence of more than one FCR in maize root PM. Thus, the low yield observed for FCR activity (Table I) demonstrates that a significant portion of that FCR activity present in whole PM was resolved from the purified enzyme reported herein, and would not be released from the affinity column at less than 1 mM NADH (13). This was further substantiated by the fact that the ratio of total FCR:DQR activities in the purified enzyme preparation was decreased approximately threefold from the ratio measured in whole PM fractions, and by the decreased degree of purification exhibited by FCR as compared to DQR. Also, some FCR may have been inactivated during chromatography, contributing to the low yields.

Similarly, the differences in specificity for reduced pyridine nucleotide substrates observed between the DQR measured in whole PM fractions (NADPH:NADH activity ratio = 0.57) (13); and the purified enzyme (NADPH:NADH activity ratio = 0.78) (Table II) suggested that more than one DQR species was present in maize root PM and resolved upon affinity purification. Alternately, the shift in donor specificity could have been caused by altered enzyme conformation from delipidation by detergent.

The higher preference of juglone reductase for NADPH was unexpected considering the fact that other acceptor substrate-associated activities utilized NADH and NADPH at nearly equal rates. The different acceptor specificity for juglone-dependent activity may reflect one or more of several interrelated events occurring on the enzyme. For example, certain hydroxy-substituted napthoquinones act as mitochondrial electron transport-associated sulfhydryl group antagonists (19). Thus juglone could act as an inhibitor of NADH-dependent activity, which was clearly sensitive to another sulfhydryl inhibitor, PCMPS (Table III), while simultaneously acting as an electron acceptor for the same activity.

The specific stimulation by FMN but not FAD following prolonged incubation of the flavin with purified enzyme which had been isolated without flavins in the column buffers suggests that the native enzyme requires a FMN cofactor for electron transport activity. The extended incubation times required for activation of the enzyme by FMN suggest that the putative flavin cofactor is noncovalently bound to the enzyme. However, it should be noted that the enzyme fraction which was purified without flavin was only about one-half to one-third as active as preparations isolated with flavins included in column buffers. The actual flavin content must be determined in order to demonstrate that the enzyme is indeed a flavoprotein.

We have purposefully excluded from this communication any measurements of kinetic parameters for the purified reductase. Activity changes upon detergent solubilization have been noted, particularly with regard to DQR activity (13). Similarly, detergent-induced shifts in kinetic parameters have been reported for a membrane bound reductase isolated from glyoxysomal membranes (15). Thus, gross changes in protein conformation may occur upon delipidation of the PM protein by Triton X-100 and subsequent inclusion of the protein into detergent micelles. Measurements of kinetic parameters have been obtained with PM preparations, resulting in apparent K_m for NADH of 24 μM, using ferricyanide as the electron donor (5). This probably represents an estimate for a mixture of FCR species. Elution of the detergent-solubilized reductase

from dye-ligand affinity columns occurred at a concentration point in the NADH gradient between 5 to 10 μM NADH (Fig. 1). This may represent an approximate range of values for the K_m of the solubilized enzyme with regard to NADH. This estimate must be considered as preliminary, sine the NADH concentration in gradient fractions was calculated by extrapolation between fractions at the initiation of the gradient and at 20 μM NADH, due to difficulty in measurement of absorbance at 340 nm in fractions containing less than 20 μM NADH. More precise estimates of kinetic parameters for the purified enzyme will be obtained when sufficient enzyme protein is available for reconstitution with phospholipids to regain native conformation.

At present, the physiological function of PM electron transport proteins in roots of graminaceous monocots, including the 27 kD maize protein, is unknown. The data regarding electron donor and acceptor specificities discussed above emphasize the need for further purification, resolution, and characterization of the respective electron transport activities present in PM. Subsequent research must concentrate on reconstitution of the purified protein(s) into phospholipid vesicles for kinetic analysis, identification of possible associated redox chain components and analysis of potential physiological primary electron acceptor(s).

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